REMARKS/ARUGMENTS

Upon entry of this amendment, claims 20-25 will be canceled without prejudice or disclaimer so that claims 1-12 and 20-25 will be canceled claims; claims 13, 15, 17, 18 and 19 will be amended; and claims 26-28 will be added. Claims 13-19 and 26-28 will be pending with claims 13, 15, 17-19 being independent claims.

The pending claims have been amended herein to clarify that the methods in the pending claims are performed *in vitro*.

Reconsideration and allowance of the application are respectfully requested.

Consideration of Supplemental Information Disclosure Statement And Submission Of Third Supplemental Information Disclosure Statement

Applicants express appreciation for the inclusion with the Office Action of initialed copies of the Forms PTO-1449 submitted with the Supplemental Information Disclosure Statement filed September 28, 2005, whereby the Examiner's consideration of the Supplemental Information Disclosure Statement is of record.

Applicants are submitting on even date herewith a Third Supplemental Information Disclosure Statement, and request that the Examiner forward an initialed copy of the Form PTO-1449 submitted therewith with the next communication from the Patent and Trademark Office.

Response To Prior Art Rejections

(a) Claims 13-25 are rejected under 35 U.S.C. 102(b) as being anticipated by Welter et al., U.S. Patent No. 4,418,069, or Dereu et al. U.S. Patent No. 4,730,053, or EP 0 366,990 or CA 0227898 or WO 97/26968.

In response to these grounds of rejection, Applicants note that claims 13, 15 and 17-19 have been amended to clarify that the methods are performed by combining at least the recited components *in vitro*, and claims 20-25 have been canceled.

Accordingly, the grounds of rejection set forth in the Office Action are without appropriate basis in that each and every feature recited in Applicants' claims is not disclosed in the cited documents, and the grounds of rejection should be withdrawn. For example, the rejections contend that each of Welter et al., Dereu et al., EP 0 366,990, CA 0227898 and WO 97/26968 discloses *in vivo* administration. However, it does not appear that any of Welter et al., Dereu et al., EP 0 366,990, CA 0227898 or WO 97/26968 discloses *in vitro* combining of ingredients as recited in Applicants' claims.

Applicants note that Dereu et al. discloses *in vitro* experiments starting at the bottom of column 4; however, these experiments (which apparently measure mercaptone concentration by means of Ellmans Reagent, as disclosed in Dereu et al. beginning at column 4, line 33, do not appear to teach Applicants' recited methods. Applicants note that amongst other benefits associated with Applicants' methods, there is provided the advantageous capability of determining useful substrates.

Accordingly, the rejections of record should be withdrawn.

(b) Claims 13-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over combined teachings of Bjornstedt et al. (JBC, 1995), Kumar et al. (Eur. J. Biochem. 1992)) and Arteel et al. (hereinafter "Arteel") (Chem. Res. Toxic., Vol. 12, 1999, pages 264-269).

In this ground of rejection, it is contended that "Bjornstedt et al teach a method for reduction of a substrate with thioredoxin reductase, comprising combining the thioredoxin reductase, the substrate and NAPDH under conditions to reduce the substrate, the substrate being Selenocystein instead of compounds claims in herein and one of them being ebselen, see page 11761."

Initially, in response to this assertion, it appears that "Selenocystein" in the above quoted portion of the rejection should be "selenocystine".

Moreover, Applicants note that, as disclosed in Bjornstedt at page 11761, right column, third full paragraph, Bjornstedt is directed to the investigation of whether thioredoxin reductase and thioredoxin can reduce lipid hydroperoxides and if low molecular weight selenium compounds could act as charge transfer catalysts. Bjornstedt discloses that this could be an important alternative pathway for the detoxification of hydroperoxides in addition to GSH-Px-mediated reduction. Thus, as noted in the rejection, Bjornstedt does not disclose methods as recited in Applicants' claims which include the recited compounds let alone teach or suggest that compounds having a structure such as ebselen would be a substrate, an inhibitor or most likely not show any reactivity. In fact, ebselen is not a substrate for a majority of the world's thioredoxin reductases found in all bacteria, plants, yeast, etc. These thioredoxin reductases do not reduce ebselen and, in fact, ebselen acts as an inhibitor.

Regarding Kumar, Kumar relates to the fact that sodium selenite is a redox cycling agent, and does not disclose methods as recited in Applicants' claims which include the recited compounds let alone teach or suggest that compounds having a structure such as ebselen would be a substrate, an inhibitor or most likely not show any reactivity.

The rejection appears to attempt to overcome the deficiencies of Bjornstedt and Kumar by relying of the disclosure of Arteel. In particular, the rejection contends that, "Arteel is teaching that activity of selenocystein and ebselen are similar when oxidation of thioredoxin is concerned that is they get reduced in the same manner, and it will be expected that selenite and ebselen will behave the same way, see page 264, because selenium is known to be reducing agent in the chemical art. Thus claimed method is no more than a mere combination and variation of prior art teachings, absent evidence to the contrary."

Applicants submit that the deficiencies of Bjornstedt and Kumar are not overcome by Arteel. One having ordinary skill in the art would not have combined the disclosures to arrive at Applicants' claimed subject matter. Moreover, any combination of the documents would not arrive at Applicants' claims.

Applicants submit that the rejection is without appropriate as the rejection does not set forth any *prima facie* case of obviousness. The rejection merely contends that the claimed method, without even referring to any of the methods recited by Applicants, is not more than a mere combination and variation of prior art teachings, absent evidence to the contrary. The rejection does not set forth any indication as to how the documents are being combined and/or varied. In fact, the rejection does not indicate

what is intended by "a mere combination and variation of prior art teachings". The Examiner is reminded that a rejection must determine the scope and contents of the prior art; ascertain the differences between the prior art and the claims in issue; resolve the level of ordinary skill in the pertinent art; and evaluate evidence of secondary considerations. Moreover, there must be an expectation of success. Thus, if the rejection is maintained, the Examiner is respectfully requested to clarify the rejection to set for its basis.

Applicants remind that Examiner that Arteel does not teach any methods wherein ebselen is used as a substrate for thioredoxin reductase. Arteel performs experiments with respect to the activity of mammalian thioredoxin reductase using a peroxynitrite reductase. Thus, at page 264, at the bottom of the left-hand column, Arteel discloses, "Here we investigated whether mammalian TR [thioredoxin reductase] can function as a peroxynitrite reductase." In performing the study, as disclosed in the Results section at page 265, left-hand column, Arteel infuses peroxynitrite to maintain a 0.2 μM steady-state concentration in potassium phosphate buffer. Arteel uses benzoate hydroxylation and nitrite formation as indices of oxidation reactions of peroxynitrite and of peroxynitrite reduction. Arteel particularly notes that when selenocystine or ebselen are present in the reaction mixture, there is a significant suppression of benzoate hydroxylation and an increase in nitrite formation until the NAPDH was oxidized. Arteel particularly specifies that <u>the addition of thioredoxin did not enhance these effects</u> (page 264, in the Abstract). Moreover, on page 265, right-hand column, Arteel discloses that. "Addition of TR to ebselen had no effect under these conditions (▼)."

Therefore, Arteel should be considered to be nonenabling for processes wherein thioredoxin is present as Arteel does not teach or suggest any need for having the thioredoxin present in the reaction.

Applicants further note that as discussed in its previous responses the prior art at most teaches that ebselen is an inhibitor of thioredoxin reductase, and that ebselen selenoxide can be a substrate. However, Applicants' claims do not include ebselen selenoxide. Arteel shows no effect of thioredoxin on reduction of ebselen selenoxide by NADPH and thioredoxin reductase.

In contrast to the prior art of record, the present invention recognizes and demonstrates that ebselen is a substrate being reduced by NADPH and thioredoxin reductase with a low Km-value meaning that it is a very good substrate undergoing unlimited cycles of oxidation/reduction in the presence of hydrogen peroxide without affecting the activity of the enzyme. The reduced ebselen is called ebselen selenol and has the Se-N bond broken by reduction. The selenol is oxidized back to ebselen by hydrogen peroxide or another peroxide and a new cycle starts. The reaction is ultimately driven by NADPH. Reduced thioredoxin strongly enhances the thioredoxin reductase reaction which is also proven by determination of the rate of reduction of ebselen by reduced thioredoxin using kinetics with tryptophan fluorescence. The result, never seen before, is that ebselen is a very efficient oxidant of reduced thioredoxin.

Thus, Applicants submit that it is by no means clear that ebselen would be a substrate for thioredoxin or thioredoxin reductase following any disclosure in Arteel, or any of the other documents utilized in the rejection. The substrate used in Arteel's work is selenocysteine, which is different from the selenocystine of Bjornstedt.

Moreover, the assertion in the rejection that selenium is a reducing agent in the chemical art is a not clear. In fact, selenite is an oxidizing agent, but sulfide is a reducing agent in the chemical art.

Applicants respectfully submit that one having ordinary skill in the art would not have found it obvious to have combined the disclosures to arrive at the claimed subject matter. There is no teaching or suggestion in the documents utilized in the rejection that mammalian thioredoxin reductase is a selenoenzyme and there is therefore no possibility of deducing that ebselen would be a substrate. In fact, most people testing thioredoxin reductase at the time Applicants' invention would buy the bacterial enzyme, which was commercially available from IMCO in Stockholm as the only source and they would have seen no reactions with ebselen. Applicants' experiments utilized preparations of mammalian thioredoxin reductase from human placenta or calf thymus and with that Applicants observed that ebselen is a substrate. An extremely fast reaction with thioredoxin was part of Applicants' findings.

Accordingly, the rejection of record is without appropriate basis, and should be withdrawn.

CONCLUSION

In view of the foregoing, the Examiner is respectfully requested to reconsider and withdraw the rejections of record, and allow each of the pending claims.

Applicants therefore respectfully request that an early indication of allowance of the application be indicated by the mailing of the Notices of Allowance and Allowability.

Should the Examiner have any questions regarding this application, the Examiner is invited to contact the undersigned at the below-listed telephone number.

Respectfully submitted,
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